Dkt. 60795-A/JPW/ADM/PL

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Marie Sudam Pathirana

U.S. Serial No.: Not Yet Known

Filed : Herewith

For : DNA Encoding Orphan SNORF68 Receptor

1185 Avenue of the Americas
New York, New York 10036
January 16, 2002

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

PRELIMINARY AMENDMENT AND INFORMATION DISCLOSURE STATEMENT

Please amend the subject application as follows:

In the Specification:

On page 1, line 5, please insert the following as a separate paragraph:

--This application is a continuation-in-part of U.S. Serial No. 09/466,570, filed December 17, 1999, the contents of which are herein incorporated by reference.--

Please replace the paragraph beginning on page 3, line 3, with the following paragraph:

--This invention provides a recombinant nucleic acid comprising a nucleic acid encoding a mammalian SNORF68 receptor, wherein the mammalian receptor-encoding nucleic acid hybridizes under high stringency conditions to a nucleic acid encoding a human SNORF68

receptor and having a sequence identical to the sequence of the human SNORF68 receptor-encoding nucleic acid contained in plasmid pEXJ.T3T7-hSNORF68-f (ATCC Patent Deposit Designation PTA-1041).--

Please replace the paragraph beginning on page 5, line 3, with the following paragraph:

--This invention provides a recombinant nucleic acid comprising a nucleic acid encoding a mammalian SNORF68 receptor, wherein the mammalian receptor-encoding nucleic acid hybridizes under high stringency conditions to a nucleic acid encoding a human SNORF68 receptor and having a sequence identical to the sequence of the human SNORF68 receptor-encoding nucleic acid contained in plasmid pEXJ.T3T7-hSNORF68-f (ATCC Patent Deposit Designation PTA-1041).--

Please replace the paragraph beginning on page 5, line 26, with the following paragraph:

--The plasmid pEXJ.T3T7-hSNORF68-f was deposited on December 8, 1999, with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded Patent Deposit Designation No. PTA-1041.--

Please replace the paragraph beginning on page 25, line 20 through page 26, line 2 with the following paragraph:

--This invention also provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with

a nucleic acid encoding a mammalian orphan receptor, wherein the probe has a sequence corresponding to a unique sequence present within one of the two strands of the nucleic acid encoding the mammalian orphan receptor and is contained in plasmid pEXJ.T3T7-hSNORF68-f (ATCC Patent Deposit Designation PTA-1041). This invention also provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian orphan receptor, wherein the probe has a sequence corresponding to a unique sequence present within (a) the nucleic acid sequence shown in Figure 1A-1C (SEQ ID NO: 1) or (b) the reverse complement thereto. In one embodiment, the nucleic acid is DNA. In another embodiment, the nucleic acid is RNA.--

On page 26, line 22, please insert the following new paragraphs:

--This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

Experimental Details

Localization of RNA coding for human SNORF68 receptor

Materials and Methods

Quantitative PCR using a fluorogenic probe with real time detection: Quantitative PCR using fluorogenic probes used to characterize the distribution of SNORF68 RNA. This assay utilizes two oligonucleotides for conventional PCR amplification and a third specific oligonucleotide probe that is labeled with a

reporter at the 5' end and a quencher at the 3' end of the oligonucleotide. In the instant invention, FAM (6-carboxyfluorescein) was used as the reporter, and BH1 (Biosearch) was used as the quencher. As amplification progresses, the labeled oligonucleotide probe hybridizes to the gene sequence between the two oligonucleotides used for amplification. The nuclease activity of *Taq* thermostable DNA polymerase is utilized to cleave the labeled probe. This separates the quencher from the reporter and generates a fluorescent signal that is directly proportional to the amount of amplicon generated. This labeled probe confers a high degree of specificity. Non-specific amplification is not detected as the labeled probe does not hybridize and as a consequence is not cleaved. All experiments were conducted in a PE7700 Sequence Detection System (PE Biosystems, Foster City CA),

<u>Quantitative RT-PCR:</u> Quantitative RT-PCR was used for the detection of SNORF68 RNA.
For use as a template in quantitative PCR reactions, cDNA was synthesized by reverse transcription from total human RNA. Reverse transcription by SuperScriptII RNAse H^- and (GibcoBRL/life Technologies) was primed using random hexamers. Parallel reactions included ^{32}P labeled dCTP to allow quantification of the cDNA. Following reverse transcription, cDNA was phenol/chloroform extracted and precipitated. Incorporation of ^{32}P dCTP was assessed after precipitation with trichloroacetic acid and the amount of cDNA synthesized was calculated.

For PCR reactions, primers with the following oligonucleotide sequences were used:

Forward primer hSN68-1124F had a sequence which began with 5'-T

located at nucleotide number 1149 and ended at with G-3' at nucleotide number 1164. (See Figures 1A-1C.)

Reverse primer hSN68-1203R had a sequence complementary to the sequence which began at 5'-G located at nucleotide number 1210 and ending with C-3' at nucleotide number 1228. (See Figures 1A-1C.)

Fluorogenic oligonucleotide probe hSN68-1158T had a sequence which began with 5'(6-FAM)-C located at nucleotide number 1183 and ended at with A-(TAMRA)3' at nucleotide number 1205. (See Figures 1A-1C.)

Using this primer set, amplicon length is 80 bp for SNORF68. Each PCR reaction contained 3.0 ng cDNA. Oligonucleotide concentrations were: 500 nM of forward and reverse primers, and 200 nM of fluorogenic probe. PCR reactions were carried out in 50 ml volumes using TaqMan universal PCR master mix (PE Applied Biosystems). Buffer for RT-PCR reactions contained a fluor used as a passive reference (ROX: Perkin Elmer proprietary passive reference I). All reagents for PCR (except cDNA and oligonucleotide primers) were obtained from Perkin Elmer (Foster City, CA). Reactions were carried in a PE7700 sequence detection system (PE Applied Biosystems) using the following thermal cycler profile: 50EC 2 min., 95EC 10 min., followed by 40 cycles of: 95EC, 15 sec., 60EC 1 min.

Standard curves for quantification of human SNORF68 were constructed using genomic DNA. Negative controls consisted of mRNA blanks, as well as primer and mRNA blanks. To confirm that the mRNA was not contaminated with genomic DNA, PCR reactions were carried out without reverse transcription using Taq DNA polymerase. Integrity of RNA was assessed by amplification of

RNA coding for cyclophilin or glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Following reverse transcription and PCR amplification, data was analyzed using PE Biosystems sequence detection software. The fluorescent signal from each well was normalized using an internal passive reference, and data was fitted a standard curve to obtain relative quantities of SNORF68 expression.

Results

Detection of mRNA coding for human SNORF68 receptor: mRNA was isolated from multiple tissues (Table 1) and assayed as described.

High levels of mRNA encoding SNORF68 receptor in testes, with relatively lower expression in most of the other regions assayed, implicates a role for this receptor in reproductive function and/or regulation of steroid hormones.

In addition to the potential therapeutic applications identified in Table 1, the localization data for mRNA encoding the human SNORF68 receptor indicates that the DNA encoding the human SNORF68 receptor can be used to predict the likelihood that a tissue sample of unknown tissue origin is of testes origin with respect to a given individual. In addition, with respect to a given individual, one could determine whether a given tissue sample of unknown origin is of testes origin as opposed to having the origin of another tissue, e.g. the liver, spleen, or pancreas. Such determinations may be used for various purposes including but not limited to the detection of tumor metastasis.

Table 1

Distribution of mRNA coding for human SNORF68 receptor using qRT-PCR

mRNA encoding SNORF68 is expressed as copies/ng cDNA

Region	**h-SNORF68**	**Potential therapeutic applications**
adrenal gland (whole)	1813	Regulation of metabolic steroids, regulation of epinephrine release
amygdala	2043	Depression, phobias, anxiety, mood disorders
cerebellum	4402	Motor coordination
cerebral cortex	1152	Cognition, sensory and motor integration
dorsal root ganglia	1648	Sensory transmission
heart	314	Cardiovascular disorders
hippocampus	2337	Cognition/memory
hypothalamus	1884	Appetite/ obesity, neuroendocrine regulation
kidney cortex	2148	Electrolyte balance, hypertension
kidney medulla	1656	Electrolyte balance, hypertension
liver	88	Diabetes
lung	936	Respiratory disorders, asthma
medulla	1584	Sensory transmission
pancreas	140	Diabetes, endocrine disorders
pituitary (whole)	1787	Endocrine/ neuroendocrine regulation
pontine reticular formation	1283	Regulation of somatosensory, motor, visual, auditory, autonomic and affective processes
prostate	921	Urinary dysfunction
skeletal muscle	487	Musculoskeletal disorders
small intestine	547	Gastrointestinal disorders
spinal cord lumbar	754	Analgesia, sensory modulation and transmission
spleen	78	Immune disorders
stomach	340	Gastrointestinal disorders

testes	9679	Reproductive function, regulation of steroid hormones
thalamus	1002	Sensory integration disorders
uterus	1251	Reproductive function, regulation of steroid hormones

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In the Claims:

Please cancel claim 1 without disclaimer or prejudice to applicants' right to pursue the subject matter of this claim in a future continuation or divisional application.

Please amend claim 2 as follows:

--2. (Amended) A recombinant nucleic acid comprising a nucleic acid encoding a human SNORF68 receptor, wherein the human SNORF68 receptor comprises an amino acid sequence identical to the sequence of the human SNORF68 receptor encoded by the nucleotide sequence beginning at the start codon at positions 62-64 and ending at the stop codon at positions 1508-1510 as indicated in Figures 1A-1C (SEQ ID NO: 1).--

Please add new claim 3 as follows:

--3. (New) A recombinant nucleic acid comprising a nucleic acid encoding a human SNORF68 receptor, wherein the receptor has a sequence identical to the sequence of the human SNORF68 receptor encoded by plasmid pEXJ.T3T7-hSNORF68-f (ATCC Patent Deposit

Designation PTA-1041).--

In the Abstract of the Disclosure:

Please replace the paragraph beginning on page 31, line 5, with the following paragraph:

--This invention provides a recombinant nucleic acid comprising a nucleic acid encoding a mammalian SNORF68 receptor, wherein the mammalian receptor-encoding nucleic acid hybridizes under high stringency conditions to a nucleic acid encoding a human SNORF68 receptor and having a sequence identical to the sequence of the human SNORF68 receptor-encoding nucleic acid contained in plasmid pEXJ.T3T7-hSNORF68-f (ATCC Patent Deposit Designation PTA-1041). This invention further provides a recombinant nucleic acid comprising a nucleic acid encoding a human SNORF68 receptor, wherein the human SNORF68 receptor comprises an amino acid sequence identical to the sequence of the human SNORF68 receptor encoded by the shortest open reading frame indicated in Figures 1A-1C (SEQ ID NO: 1).--

A marked-up copy of the amendments is attached herewith as **Exhibit 1**.

REMARKS

Claims 1 and 2 were pending in the subject application. By this Preliminary Amendment, applicant has amended the specification, Abstract of the Disclosure and claim 2; canceled claim 1 without prejudice; and added new claim 3. Accordingly, upon entry of this Preliminary Amendment, claims 2 and 3 will be pending and under examination.

Support for the amendments to the specification and the Abstract of the Disclosure to correct ATCC deposit information may be found in the ATCC Deposit Receipt attached hereto as **Exhibit 3.** Applicant maintains that this Amendment raises no issue of new matter.

Support for amended claim 2 may be found inter alia in the specification, as originally-filed, on page 4, lines 3-10 and Figures 1A-1C. Support for new claim 3 may be found inter alia in the specification, as originally-filed, on page 5, lines 3-12; and page 5, lines 26-33. Applicant maintains that this Amendment raises no issue of new matter and is fully supported by the specification as filed.

Accordingly, applicants respectfully request that this Amendment be entered.

Sequence Listing

The Sequence Listing in the subject application is identical to that filed with the parent of the subject application, U.S. Serial No. 09/466,570, filed on December 17, 1999. Applicant is filing as part of the subject application copies of the paper copy of the Sequence Listing and Statement in Accordance With 37 C.F.R. §1.821(f) which were filed with U.S. Serial No. 09/466,570 on December 17, 1999. The computer readable form in the subject application is identical to that filed in U.S. Serial No.09/466,570, filed on December 17, 1999. In Accordance with 37 C.F.R. §1.821(e), please use the computer readable form filed in U.S. Serial No. 09/466,570 as the computer readable form for the instant application. It is understood that the Patent and Trademark Office will make the necessary change in application number and filing date for the sequence listing that will be used for the instant application.

Information Disclosure Statement

In accordance with the duty of disclosure under 37 C.F.R. § 1.56, applicant would like to direct the Examiner's attention to the following references which are listed on the attached Form PTO-1449 (**Exhibit 2**) and were previously cited in connection with prosecution of U.S. Serial No. 09/466,570, filed December 17, 1999; the subject application claims priority under 35 U.S.C. §120 of the filing date of that application. According to 37 C.F.R. §1.98(d), copies of patents or publications that were previously cited by, or submitted to, the Patent Office in connection with such prior applications need not accompany the Information Disclosure Statement. Accordingly, copies of the following references are not attached to this Information Disclosure Statement:

1. Bonaldo, M.F., Lennon, G. and Soares, M.B., "Normalization and Subtraction: Two Approaches to Facilitate Gene Discovery," *Genome Res.* (1996) **6(9)**, 791-806;

2. Expressed Sequence Tags Database Accession No. AI537485, National Cancer Institute, Cancer Genome Anatomy Project, (Published March 18, 1999);

3. Expressed Sequence Tags Database Accession No. AI854212, "Normalization and Subtraction: Two Approaches to Facilitate Gene Discovery," Bonaldo, et al., (Published July 15, 1999);

4. Expressed Sequence Tags Database Accession No. AA634446, Hillier, et al., (Published October 22, 1997);

5. GebEMBL Database Accession No. M76676, "The Human leukocyte platelet-activating factor receptor: cDNA epitope-bearing

analog," Kunz, et al., (Published April 27, 1993);

6. GebEMBL Database Accession No. G07162, Hudson, T., (Published June 14, 1995);

7. Kunz, D, et al., " The Human Leukocyte Platelet-activating Factor Receptor," *J. Biol. Chem.* (1992) **267:** 9101-9106;

8. Kunz, D, et al., Database GenEMBL, Accession No. M76676 (1998);

9. Skolnick, J., et al., "From genes to protein structure and function: novel applications of computational approaches in the genomic era," *Trends in Biotechnology*, (1998) 18(1): 34-39; and

10. Swiss ProtPlus Database Accession No. Q14968, "The Human Leukocyte Platelet-activating Factor Receptor. cDNA cloning, cell surface expression, and construction of a novel epitope-bearing analog," Kunz, et al., (Published November 1, 1996).

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicant's undersigned attorney invites the Examiner to telephone him at the number provided below.

No fee, other than the $370.00 fee for filing the subject application is deemed necessary in connection with the filing of this Preliminary Amendment and Information Disclosure Statement. However, if any additional fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully submitted,

John P. White
Registration No. 28,678
Attorney for Applicants
Cooper & Dunham LLP
1185 Avenue of the Americas
New York, New York 10036
(212) 278-0400

60795-A/JPW/ADM/PL

Marked-up Version of Amendments

Additions to the text are indicated by double underlining; deletions are indicated by square brackets.

In the Specification:

The replacement paragraph beginning on page 3, line 3:

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The replacement paragraph beginning on page 5, line 3:

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In the Claims:

--2. (Amended) A recombinant nucleic acid comprising a nucleic acid encoding a human SNORF68 receptor, wherein the human SNORF68 receptor comprises an amino acid sequence identical to the sequence of the human SNORF68 receptor encoded by the nucleotide sequence beginning at the start codon at

positions 62-64 and ending at the stop codon at positions 1508-1510 [shortest open reading frame] as indicated in Figures 1A-1C (SEQ ID NO: 1).--

In the Abstract of the Disclosure:

The replacement paragraph beginning on page 31, line 5:

--This invention provides a recombinant nucleic acid comprising a nucleic acid encoding a mammalian SNORF68 receptor, wherein the mammalian receptor-encoding nucleic acid hybridizes under high stringency conditions to a nucleic acid encoding a human SNORF68 receptor and having a sequence identical to the sequence of the human SNORF68 receptor-encoding nucleic acid contained in plasmid pEXJ.T3T7-hSNORF68-f (ATCC Patent Deposit Designation No. PTA-1041 [____]). This invention further provides a recombinant nucleic acid comprising a nucleic acid encoding a human SNORF68 receptor, wherein the human SNORF68 receptor comprises an amino acid sequence identical to the sequence of the human SNORF68 receptor encoded by the shortest open reading frame indicated in Figures 1A-1C (SEQ ID NO: 1).--